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ORIGINAL ARTICLE

Isolation and identification of ochratoxin A-producing *Aspergillus* section *Nigri* strains from California raisins

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A. niger, *Aspergillus carbonarius*, ochratoxin, raisins, vineyards.

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Abstract

Aims: To determine incidence and levels of ochratoxin A (OTA) in California raisins and to isolate and characterize OTA-producing fungi from California raisin vineyard populations.
Methods and Results: Forty raisin clusters sampled from four California vineyards in the San Joaquin Valley were analysed for OTA content using immunoaffinity and HPLC methods. OTA was detected in 93% of the samples, at levels from 0·06 to 11·4 ng g^{-1}. From these raisin samples, a total of 400 strains of *Aspergillus* were isolated and analysed for OTA production. Twelve isolates (3%), from five raisin samples, produced OTA. These isolates were identified as *Aspergillus carbonarius*, based on morphological characteristics and multilocus sequence analysis. Levels of OTA produced by these isolates on raisin agar ranged from 0·9 to 15 $\mu g\ g^{-1}$.
Conclusions: OTA is a common contaminant of raisin vineyards, but average levels are much lower than EU regulatory limits for dried fruit. The primary species responsible for OTA contamination in California raisins is *A. carbonarius*.
Significance and Impact of the Study: This study illustrates that low-level OTA contamination of raisins occurs in California and that ecological studies of *A. carbonarius* within the *Aspergillus* section *Nigri* population on raisins are warranted to monitor ochratoxigenic potential of the crop.

Ochratoxin A (OTA) is a polyketide mycotoxin produced by several species of *Aspergillus* and *Penicillium* (Pitt 1987; Abarca *et al.* 1994; Teren *et al.* 1996; Varga *et al.* 1996; Bayman *et al.* 2002). OTA is a public health concern because it is nephrotoxic, carcinogenic, teratogenic, immunosuppressive and cytotoxic (Haubeck *et al.* 1981; Petzinger and Ziegler 2000; Follmann and Lucas 2003; Kamp *et al.* 2005). OTA contamination has been associated with several food products, including cereal grains, beer, wine, grapes, coffee, and dried fruit and nuts (Abarca *et al.* 1994; MacDonald *et al.* 1999; Bayman *et al.* 2002; Sage *et al.* 2002; Batista *et al.* 2003; Lund and Frisvad 2003; Magnoli *et al.* 2003; Serra *et al.* 2003; Taniwaki *et al.* 2003). This has resulted in maximum allowable limits being set in the European Union (EU) for OTA in many of these commodities (Anon 2006). In raisins and other dried vine fruit, the limit has been set at 10 ng g^{-1} (parts per billion, ppb). Similar regulatory limits have been proposed in Canada (Website: http://www.hc-sc.gc.ca/fn-an/alt_formats/hpfb-dgpsa/pdf/consultation/myco_consult_ochra-eng.pdf, accessed 7 September, 2010). In contrast, no such regulatory limits have been set by the US Food and Drug Administration.

In raisins and grapes, the major OTA-producing fungi belong to *Aspergillus* section *Nigri*, which include *Aspergillus niger*, *Aspergillus carbonarius* and *Aspergillus tubingensis*. OTA production by black-spored Aspergilli has been investigated in several grape- and winegrowing regions, including Spain (Bau *et al.* 2005), France (Sage *et al.* 2002), Italy (Battilani *et al.* 2006), Portugal (Serra *et al.* 2003), Argentina (Magnoli *et al.* 2003) and Australia (Leong *et al.* 2006). In all of these studies, the majority, if not all, of the ochratoxigenic isolates were identified as *A. carbonarius*, while very few isolates of *A. niger* or other

black *Aspergillus* species produced OTA. Dried vine fruits (raisins, currants, sultanas) are at even greater risk for OTA contamination, because the relative proportion of *A. carbonarius* within the population of black Aspergilli increases as moisture levels in the fruit decrease (Valero *et al.* 2006).

California supplies 45% of the world's raisin crop. In 2008, raisin grapes were grown on 221 000 acres in the San Joaquin Valley, comprising 99·5% of the US raisin acreage. More than 140 000 tons of raisins, valued at $300 million, were exported, primarily to EU countries. In the light of the regulatory limits placed on OTA contamination of dried fruit, it is important to evaluate OTA levels in California-grown raisins. Raisins are produced using three different methods: traditional tray-dried, continuous tray and dried-on-vine (DOV) representing 50, 40 and 10% of the industry, respectively (Stephen Vasquez, personal communication). Although only a small percentage of the industry, more producers are transitioning to DOV raisin production because of reduced labour. However, the longer drying time and higher moister content DOV fruit may result in increased OTA-producing fungi. Therefore, the goals of this study were to measure levels of OTA in raisins from California DOV vineyards and to determine the ochratoxigenic potential of *Aspergillus* section *Nigri* isolates recovered from raisins. In addition, species identification of ochratoxigenic isolates was performed to determine the relative contribution of each species to OTA contamination. This study represents the first step towards a large-scale survey of OTA levels in California raisins produced using the DOV method.

Materials and methods

Determination of OTA content in raisins

Ten raisin samples were collected from each of four distinct raisin vineyards located in Fresno County. Samples from vineyards 'F', 'B' and 'K' were cv. Fiesta, and samples from vineyard 'M' were cv. Selma Pete. Each sample consisted of one to three DOV clusters and harvested at 12–14% moisture. Raisins were removed from the stems, and 200 g were homogenized by grinding with dry ice in a Waring blender. A 50 g subsample of each was processed for OTA determination using OchraTest immunoaffinity columns (VICAM, Milford, MA, USA), according to the manufacturer's instructions. Briefly, ground raisins were blended with 100 ml of methanol : 1% sodium bicarbonate (70 : 30) for 1 min in a Waring blender and filtered through Whatman no. 2 filter paper (Whatman Inc., Piscataway, NJ, USA). Filtered extract was diluted 1 : 5 with phosphate-buffered saline (PBS) + 0·01% Tween 20 and filtered using 0·22-μm Millex-GP polyethersulfone syringe filters (Millipore, Bedford, MA, USA). Ten millilitres of diluted extracts was applied to OchraTest columns, and columns were washed once with PBS + 0·01% Tween 20 and once with distilled water. OTA was eluted in 1·5 ml of methanol and quantified by HPLC. For HPLC quantification, 20 μl of each sample was injected into an Agilent model 1100 high-performance liquid chromatography (Agilent Technologies, Inc., Santa Clara, CA, USA). Separations were performed using an Inertsil ODS-3 5 μ, 4·6 × 250 mm column (Varian, Inc., Palo Alto, CA, USA), with acetonitrile : 0·1% phosphoric acid (70 : 30) as the mobile phase at a flow rate of 1 ml min^{-1}. OTA was detected using an Agilent model 1321A fluorescence detector, with excitation and emission wavelengths of 333 and 460 nm, respectively. OTA amounts were calculated from peak areas in comparison with a standard curve constructed using 1–100 ng of authentic OTA (Sigma-Aldrich Corp., St Louis, MO, USA) per injection.

Screening for ochratoxigenic fungi from raisins

From the remainder of each sample, ten raisins were placed onto each of three dichloran-rose bengal-chloramphenicol agar containing 6% NaCl (DRBCS) and incubated at 28°C. Following growth for 3–7 days, ten colonies of black-spored *Aspergillus* were picked randomly from each raisin sample, for a total of 100 isolates per vineyard and 400 isolates in all. Fungi were transferred to potato dextrose agar for maintenance and were stored at −70°C as conidial suspensions in 0·05% Tween 20, 30% glycerol. To screen for OTA production, fungal isolates were grown on yeast extract-sucrose agar for 5 days at 28°C. Five agar plugs per plate were extracted with 1 ml of methanol as described (Bragulat *et al.* 2001) Extracts were filtered using 0·2-μm nylon syringe filters (Fisher Scientific, Santa Clara, CA, USA) and analysed for OTA by HPLC as described earlier.

Species identification of *Aspergillus* isolates

Twelve ochratoxigenic isolates and 12 randomly selected nonochratoxigenic isolates were transferred to Czapek yeast extract agar (CYA), CYA with 20% sucrose (CY20S), malt extract agar and Czapek-Dox solution agar (CZ). Each plate was inoculated with three 5-μl drops of freshly prepared conidial suspension (*c.* 10^5 conidia per ml in 0·05% Tween 80), incubated at 25 or 37°C for 7 days and observed for species identification by macroscopic and microscopic morphological characteristics as described in Klich (2002). Isolates were also identified at the species level using β-tubulin and calmodulin gene sequences. Genomic DNA from each sample was isolated

using a DNeasy Plant Mini kit (Qiagen, Inc., Valencia, CA, USA) with minor modifications. Fungi were inoculated in 50 ml malt extract broth with 500 μl of freshly prepared conidial suspension and incubated overnight at 25°C with shaking at 150 rev min^{-1}. Fungal cultures were harvested by centrifugation and lyophilized overnight. Lyophilized samples were crushed into a fine powder, and 20 mg of each powdered fungal sample was used as starting material for each DNA isolation reaction. Calmodulin gene fragments were PCR amplified from genomic DNA using primers CF1L (5′-GCCGACTCTTTGACYGARGAR-3′) and CF4 (5′-TTTYTGCATCATRAGYTGGAC-3′) (Peterson 2008), with the following amplification conditions: 95°C for 5 min; 35 cycles of 95°C for 30 s, 52°C for 30 s, 72°C for 1 min; and 72°C for 5 min. β-Tubulin gene fragments were PCR amplified using primers Bt2a (5′-GGTAACCAAATCGGTGCTGCTTTC-3′) and Bt2b (5′-ACCCTCAGTGTAGTGACCCTTGGC-3′) (Glass and Donaldson 1995), using the same amplification conditions as for calmodulin, except that the primer annealing temperature was 61°C. Amplified DNA fragments were purified using a Qiaquick PCR Purification kit (Qiagen), sequenced with the same primers using BigDye v 3.1 Cycle Sequencing reagents (Applied Biosystems, Inc., Foster City, CA, USA) and run on a 3730 DNA Analyzer (Applied Biosystems). Sequences were aligned using Lasergene sequence analysis software (DNASTAR, Inc., Madison, WI, USA), and homologies to sequences of reference strains were determined by BLAST search.

Quantification of OTA production on raisin agar

Raisin agar consisted of 100 g l^{-1} of ground raisins, from vineyard samples that contained no detectable OTA, and 15 g l^{-1} of agar, pH 4·4. Each ochratoxigenic isolate was point inoculated with conidia onto triplicate plates and incubated at 28°C for 5 days. Four agar plugs per plate were excised as described earlier and extracted in 1 ml of methanol for 3 h. Extracts were filtered and analysed for OTA by HPLC as described earlier. OTA was quantified using the standard curve described earlier and normalized to agar plug weight. The experiment was performed twice, and differences in OTA production among strains were analysed by one-way ANOVA with Tukey's post-test using GRAPHPAD INSTAT (version 3.06; GraphPad Software, Inc., San Diego, CA, USA).

Results

Incidence and levels of OTA in raisins

Among the 40 raisin samples taken from each of four vineyards, OTA was detected in 37 of them (Table 1).

Table 1 OTA content of vineyard raisin samples

Sample number	OTA, ng g^{-1}			
	Vineyard F	Vineyard B	Vineyard K	Vineyard M
1	0·18	0·30	0·30	1·0
2	n.d.*	0·30	0·40	0·70
3	0·15	0·20	0·06	0·03
4	0·08	0·30	0·10	0·20
5	n.d.	0·08	0·30	0·10
6	0·15	0·06	0·10	0·90
7	0·15	0·10	n.d.	2·6
8	0·18	0·20	0·90	0·50
9	0·45	0·10	0·08	1·6
10	0·06	0·40	11·4	3·0
Mean	0·14	0·22	1·37	1·08
SD	0·13	0·12	3·53	1·05

OTA, ochratoxin A.
*n.d., not detected. Limit of OTA detection was 0·02 ng g^{-1}.

OTA levels were generally very low, with only one sample from vineyard K (sample 10, 11·4 ng g^{-1}) above the EU regulatory limit of 10 ng g^{-1}. Samples from vineyards F and B contained <0·5 ng g^{-1} OTA, as did eight of the ten samples from vineyard K. However, the frequency of OTA levels >0·5 ng g^{-1} was higher in vineyard M samples, potentially as a result of late-season rain prior to sampling that led to increased fungal growth and mycotoxin production. Nevertheless, the mean OTA levels recovered from each vineyard were below the EU regulatory limit.

Isolation of OTA-producing black *Aspergillus* strains

Initial efforts to isolate ochratoxigenic fungi by plating sonic washes of raisins were unsuccessful, suggesting that populations of potential OTA-producing fungi were small in these vineyard samples or that their association with raisin surfaces resulted in poor recovery in wash solutions. Direct plating of raisins onto DRBCS, in contrast, resulted in efficient recovery of *Aspergillus* strains following growth and sporulation from the surface of the raisins. A total of 400 isolates were randomly selected from raisins plated onto DRBCS and screened for OTA production (Table 2). No OTA-producing *Aspergillus* isolates were recovered from vineyards F and B, including three isolates resembling *A. ochraceus*. In contrast, OTA-producing strains were recovered from vineyards K (five isolates) and M (seven isolates). In these screens, the ochratoxigenic strains isolated from vineyard K produced considerably higher amounts of OTA than those from vineyard M, suggesting that these strains represent distinct populations of OTA producers.

Table 2 Frequency of isolation of ochratoxigenic fungi from raisin samples

Vineyard	No. of *Aspergillus* section *Nigri* isolates screened	No. of OTA-producing isolates	Isolation frequency of OTA producers, %	Range of OTA production, μg ml^{-1}*
F	98	0	0	n.d.†
B	99	0	0	n.d.
K	100	5	5	1·43–6·66
M	100	7	7	0·05–0·37
Total	397	12	3	0·05–6·66

OTA, ochratoxin A.
*Amount of OTA extracted from agar plugs of fungal cultures on YES agar.
†n.d., not detected. Limit of OTA detection was 1 ng ml^{-1}.

Species identification of ochratoxigenic and nonochratoxigenic strains

The 12 OTA-producing isolates, along with 12 OTA-nonproducing isolates, selected randomly from the 400 strains collected, were subjected to differential morphological analyses as described in Klich (2002). The most distinguishing characteristics to differentiate *A. niger* aggregate species (*A. niger*, *A. tubingensis* and *Aspergillus awamori*) from *A. carbonarius* are growth at 37°C and conidial diameter (Samson *et al.* 2007). All 12 of the ochratoxigenic isolates showed restricted growth at 37°C (Fig. 1a), consistent with *A. carbonarius*, while all of the nonochratoxigenic isolates grew well at 37°C (Fig. 1b), characteristic of *A. niger* aggregate species. This effect was more pronounced at 40°C, at which the ochratoxigenic strains did not grow and the nonochratoxigenic strains grew well (data not shown). In addition, all OTA-producing strains formed large (7–10 μm diameter), rough conidia, indicative of *A. carbonarius*, and all OTA-nonproducing strains formed smaller (<4 μm diameter), finely to coarsely rough conidia, indicative of *A. niger* aggregate species (Fig. 1c).

To confirm tentative morphological species identification, calmodulin and β-tubulin gene fragments were sequenced and analysed for DNA homology to genes

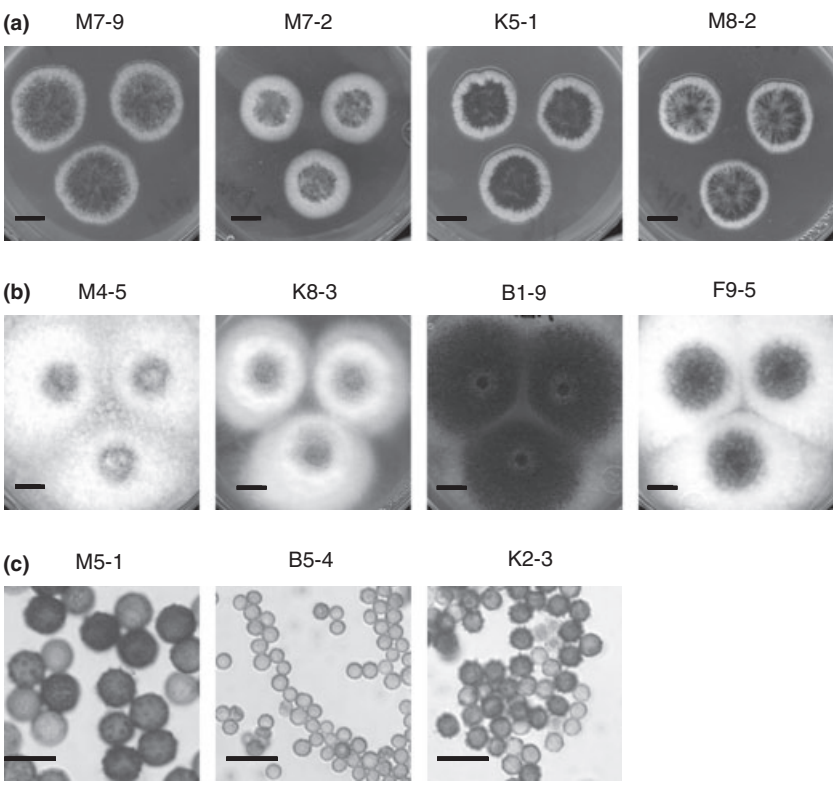

Figure 1 Morphological characteristics of *Aspergillus* section *Nigri* isolates. Growth of representative strains of (a) OTA-producing isolates and (b) OTA-nonproducing isolates on Czapek yeast extract agar at 37°C. Bar = 1 cm. (c) Micrographs of conidia from OTA-producing (strain M5-1) and OTA-nonproducing (strains B5-4 and K2-3) isolates. Bar = 10 μm. OTA, ochratoxin A.

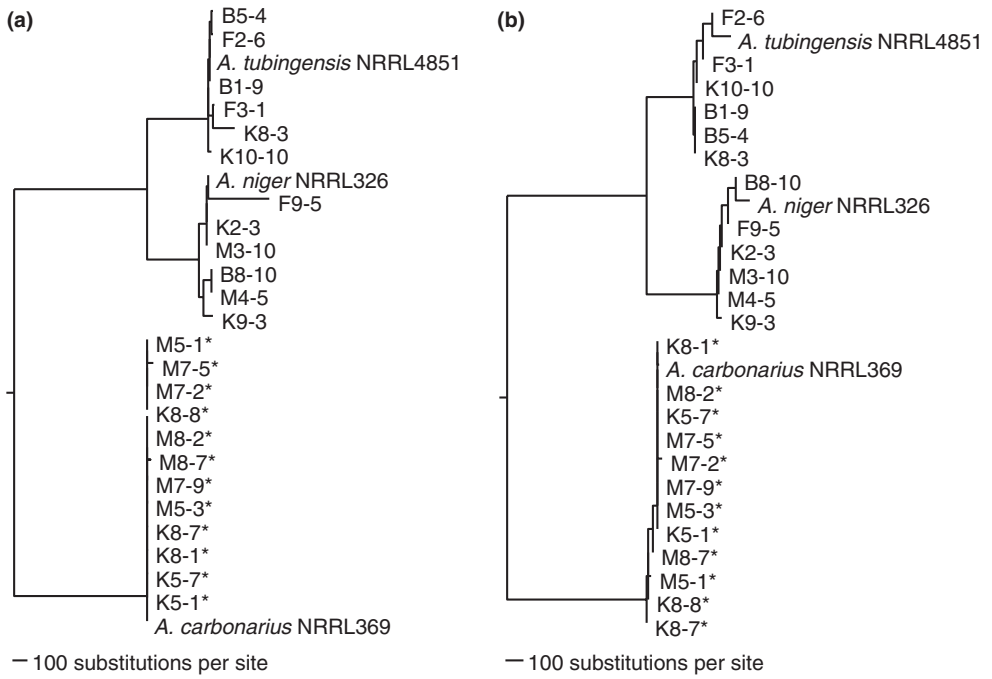

Figure 2 Phylogenetic trees of (a) calmodulin and (b) β-tubulin gene fragment sequences. Sequences were aligned using ClustalW, and trees were constructed by neighbour joining. Reference strains (*Aspergillus niger* NRRL326, *Aspergillus carbonarius* NRRL369 and *Aspergillus tubingensis* NRRL4851) were chosen based on sequence similarity to each phylogenetic group for each gene fragment analysed. Asterisks indicate OTA-producing isolates. Bar = 100 nucleotide substitutions per site. OTA, ochratoxin A.

from known species. As shown in Fig. 2, all ochratoxigenic strains, identified morphologically as *A. carbonarius*, clustered with previously classified *A. carbonarius* strains, using both calmodulin (Fig. 2a) and β-tubulin (Fig. 2b) sequences. In contrast, the 12 nonochratoxigenic strains, identified as *A. niger* aggregate species, clustered as *A. niger* (six strains) and *A. tubingensis* (six strains). The same strains clustered together with previously classified *A. niger* or *A. tubingensis* strains whether the phylogenetic trees were constructed using calmodulin or β-tubulin sequences.

OTA production on raisin agar

Raisin agar was used as a surrogate medium for whole raisins, to provide uniform nutrient conditions and solid surface for growth and OTA production by *A. carbonarius* strains. The 12 ochratoxigenic *A. carbonarius* strains tested in this study consistently produced OTA on this medium and differed among strains in the amount of OTA produced (Fig. 3). High OTA producers (strains K8-1, K8-7 and K8-8) produced over 15 $\mu g\ g^{-1}$ OTA, which was significantly higher than moderate OTA producers (strains K5-1, K5-7, M5-1 and M5-3) and low OTA producers (strains M7-2, M7-5, M7-9, M8-2 and

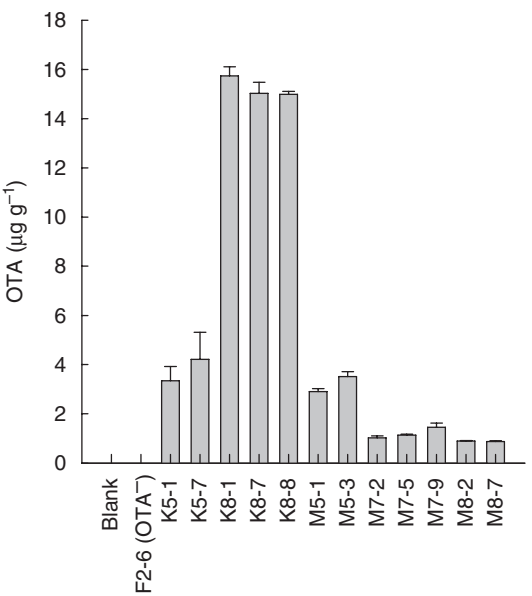

Figure 3 OTA production on raisin agar. Data points and error bars represent means and standard deviation of triplicate samples from one representative experiment. Strain F2-6 is an OTA-nonproducing *Aspergillus tubingensis* strain, included as a negative control. OTA, ochratoxin A.

M8-7) ($P < 0{\cdot}001$). Moderate OTA-producing strains yielded 3–5 $\mu g\ g^{-1}$ OTA, which was significantly higher than low OTA-producing strains (0·9–1·5 $\mu g\ g^{-1}$) ($P < 0{\cdot}01$).

Discussion

This report demonstrates that OTA is present as a contaminant in some California raisin vineyards, but at relatively low concentrations (Table 1). Of the samples analysed, all but one were well below the EU threshold limit of 10 ng g^{-1} (sample 10 from vineyard K). Still, if vineyard K samples were combined and homogenized into a composite sample, the OTA level recovered would be below the EU limit. Nevertheless, these data show the potential for OTA contamination to occur at significant levels in California raisin vineyards. While OTA was detected in nearly every vineyard sample, ochratoxigenic fungi (12 isolates) were isolated from only 5 of the 40 samples (Table 2). As black-spored Aspergilli were readily isolated from all samples, it is likely that OTA-producing strains are rare among the population as a whole and that larger numbers of isolates per sample must be screened to recover ochratoxigenic fungi from an OTA-positive sample or from an entire vineyard.

All 12 OTA-producing fungi isolated in this study were identified as *A. carbonarius* by morphological and DNA sequence analyses (Figs 1 and 2). No other *A. niger* aggregate species were identified as OTA producers. Other studies have shown that *A. carbonarius* is the dominant OTA producer in raisins. A survey of ochratoxigenic fungi from dried fruits in Argentina determined that although *A. niger* aggregate species were more frequently isolated, only 28% of them produced OTA, while 83% of *A. carbonarius* isolates produced OTA, and at much higher levels (Magnoli *et al.* 2003). Similarly, a study of OTA in Corinth raisin vineyards in Greece showed that 80% of *A. niger* isolates produced <5 ng g^{-1} of OTA, while 78% of *A. carbonarius* isolates produced >25 ng g^{-1} of OTA (Tjamos *et al.* 2004). A survey of dried vine fruits from the Spanish market resulted in isolation of 91 *A. carbonarius* strains, 88 of which were OTA producers, and 168 *A. niger* strains, only one of which produced OTA (Abarca *et al.* 2003).

Using raisin agar as a surrogate medium to simulate fungal growth and OTA production on whole raisins, the *A. carbonarius* strains in our study could be grouped as high (>15 $\mu g\ g^{-1}$), moderate (3–5 $\mu g\ g^{-1}$) and low (<1·5 $\mu g\ g^{-1}$) OTA producers. Other studies have shown that OTA production is variable within an *A. carbonarius* population (Sage *et al.* 2002; Magnoli *et al.* 2003; Tjamos *et al.* 2004; Bau *et al.* 2005; Battilani *et al.* 2006). Further work is necessary to address whether there is an ecological effect of this variability on entire *A. carbonarius* populations, or *Aspergillus* section *Nigri* populations in general, in terms of potential competitive advantage conferred by OTA production.

This study showed that OTA contamination is present in unprocessed California DOV vineyard samples. Other surveys have focused largely on processed, ready-to-eat raisins sampled from markets (Abarca *et al.* 2003; Lombaert *et al.* 2004; Magnoli *et al.* 2004; Iamanaka *et al.* 2005). Parallel surveys of Turkish sultanas found a mean OTA level of 3·4 ng g^{-1} in unprocessed sultanas (Meyvaci *et al.* 2005), but a mean OTA level of 1·4 ng g^{-1} in processed sultanas (Aksoy *et al.* 2007). With this in mind, our efforts to realistically evaluate OTA in California raisins will require examination of OTA levels at each step, from vineyard through processing and packaging, to better understand the frequency and extent of OTA contamination during raisin production under different production systems.

Acknowledgements

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References

Abarca, M.L., Bragulat, M.R., Castella, G. and Cabanes, F.J. (1994) Ochratoxin A production by strains of *Aspergillus niger* var. *niger*. *Appl Environ Microbiol* **60**, 2650–2652.

Abarca, M.L., Accensi, F., Bragulat, M.R., Castella, G. and Cabanes, F.J. (2003) *Aspergillus carbonarius* as the main source of ochratoxin A contamination in dried vine fruits from the Spanish market. *J Food Prot* **66**, 504–506.

Aksoy, U., Eltem, R., Meyvaci, K.B., Altindisli, A. and Karabat, S. (2007) Five-year survey of ochratoxin A in processed sultanas from Turkey. *Food Addit Contam* **24**, 292–296.

Anon. (2006) Commission Regulation (EC) No 1881/2006, L364. *Off J Eur Union* **49**, 5–24.

Batista, L.R., Chalfoun, S.M., Prado, G., Schwan, R.F. and Wheals, A.E. (2003) Toxigenic fungi associated with processed (green) coffee beans (*Coffea arabica* L.). *Int J Food Microbiol* **85**, 293–300.

Battilani, P., Giorni, P., Bertuzzi, T., Formenti, S. and Pietri, A. (2006) Black aspergilli and ochratoxin A in grapes in Italy. *Int J Food Microbiol* **111**, S53–S60.

Bau, M., Bragulat, M.R., Abarca, M.L., Minguez, S. and Cabanes, F.J. (2005) Ochratoxigenic species from Spanish wine grapes. *Int J Food Microbiol* **98**, 125–130.

Bayman, P., Baker, J.L., Doster, M.A., Michailides, T.J. and Mahoney, N.E. (2002) Ochratoxin production by the *Aspergillus ochraceus* group and *Aspergillus alliaceus*. *Appl Environ Microbiol* **68**, 2326–2329.

Bragulat, M.R., Abarca, M.L. and Cabanes, F.J. (2001) An easy screening method for fungi producing ochratoxin A in pure culture. *Int J Food Microbiol* **71**, 139–144.

Follmann, W. and Lucas, S. (2003) Effects of the mycotoxin ochratoxin A in a bacterial and a mammalian *in vitro* mutagenicity test system. *Arch Toxicol* **77**, 298–304.

Glass, N.L. and Donaldson, G.C. (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Appl Environ Microbiol* **61**, 1323–1330.

Haubeck, H.D., Lorkowski, G., Kolsch, E. and Roschenthaler, R. (1981) Immunosuppression by ochratoxin A and its prevention by phenylalanine. *Appl Environ Microbiol* **41**, 1040–1042.

Iamanaka, B.T., Taniwaki, M.H., Menezes, H.C., Vicente, E. and Fungaro, M.H. (2005) Incidence of toxigenic fungi and ochratoxin A in dried fruits sold in Brazil. *Food Addit Contam* **22**, 1258–1263.

Kamp, H.G., Eisenbrand, G., Schlatter, J., Wurth, K. and Janzowski, C. (2005) Ochratoxin A: induction of (oxidative) DNA damage, cytotoxicity and apoptosis in mammalian cell lines and primary cells. *Toxicology* **206**, 413–425.

Klich, M.A. (2002) *Identification of Common Aspergillus Species*. Utrecht, the Netherlands: Centraalbureau voor Schimmelcultures.

Leong, S.L., Hocking, A.D., Pitt, J.I., Kazi, B.A., Emmett, R.W. and Scott, E.S. (2006) Australian research on ochratoxigenic fungi and ochratoxin A. *Int J Food Microbiol* **111**(Suppl 1), S10–S17.

Lombaert, G.A., Pellaers, P., Neumann, G., Kitchen, D., Huzel, V., Trelka, R., Kotello, S. and Scott, P.M. (2004) Ochratoxin A in dried vine fruits on the Canadian retail market. *Food Addit Contam* **21**, 578–585.

Lund, F. and Frisvad, J.C. (2003) *Penicillium verrucosum* in wheat and barley indicates presence of ochratoxin A. *J Appl Microbiol* **95**, 1117–1123.

MacDonald, S., Wilson, P., Barnes, K., Damant, A., Massey, R., Mortby, E. and Shepherd, M.J. (1999) Ochratoxin A in dried vine fruit: method development and survey. *Food Addit Contam* **16**, 253–260.

Magnoli, C., Violante, M., Combina, M., Palacio, G. and Dalcero, A. (2003) Mycoflora and ochratoxin-producing strains of *Aspergillus* section Nigri in wine grapes in Argentina. *Lett Appl Microbiol* **37**, 179–184.

Magnoli, C., Astoreca, A., Ponsone, L., Combina, M., Palacio, G., Rosa, C.A. and Dalcero, A.M. (2004) Survey of mycoflora and ochratoxin A in dried vine fruits from Argentina markets. *Lett Appl Microbiol* **39**, 326–331.

Meyvaci, K.B., Altindisli, A., Aksoy, U., Eltem, R., Turgut, H., Arasiler, Z. and Kartal, N. (2005) Ochratoxin A in sultanas from Turkey I: survey of unprocessed sultanas from vineyards and packing-houses. *Food Addit Contam* **22**, 1138–1143.

Peterson, S.W. (2008) Phylogenetic analysis of *Aspergillus* species using DNA sequences from four loci. *Mycologia* **100**, 205–226.

Petzinger, E. and Ziegler, K. (2000) Ochratoxin A from a toxicological perspective. *J Vet Pharmacol Ther* **23**, 91–98.

Pitt, J.I. (1987) *Penicillium viridicatum*, *Penicillium verrucosum*, and production of ochratoxin A. *Appl Environ Microbiol* **53**, 266–269.

Sage, L., Krivobok, S., Delbos, E., Seigle-Murandi, F. and Creppy, E.E. (2002) Fungal flora and ochratoxin A production in grapes and musts from France. *J Agric Food Chem* **50**, 1306–1311.

Samson, R.A., Noonim, P., Meijer, M., Houbraken, J., Frisvad, J.C. and Varga, J. (2007) Diagnostic tools to identify black aspergilli. *Stud Mycol* **59**, 129–145.

Serra, R., Abrunhosa, L., Kozakiewicz, Z. and Venancio, A. (2003) Black *Aspergillus* species as ochratoxin A producers in Portuguese wine grapes. *Int J Food Microbiol* **88**, 63–68.

Taniwaki, M.H., Pitt, J.I., Teixeira, A.A. and Iamanaka, B.T. (2003) The source of ochratoxin A in Brazilian coffee and its formation in relation to processing methods. *Int J Food Microbiol* **82**, 173–179.

Teren, J., Varga, J., Hamari, Z., Rinyu, E. and Kevei, F. (1996) Immunochemical detection of ochratoxin A in black *Aspergillus* strains. *Mycopathologia* **134**, 171–176.

Tjamos, S.E., Antoniou, P.P., Kazantzidou, A., Antonopoulos, D.F., Papageorgiou, I. and Tjamos, E.C. (2004) *Aspergillus niger* and *Aspergillus carbonarius* in Corinth raisin and wine-producing vineyards in Greece: population composition, ochratoxin A production and chemical control. *J Phytopathol* **152**, 250–255.

Valero, A., Farre, J.R., Sanchis, V., Ramos, A.J. and Marin, S. (2006) Kinetics and spatial distribution of OTA in *Aspergillus carbonarius* cultures. *Food Microbiol* **23**, 753–756.

Varga, J., Kevei, E., Rinyu, E., Teren, J. and Kozakiewicz, Z. (1996) Ochratoxin production by *Aspergillus* species. *Appl Environ Microbiol* **62**, 4461–4464.